

COPY OF ALL CLAIMS

12. (currently amended) A method for generating new catalytic activity in an enzyme, comprising the steps of:

- a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red or into a functional derivative thereof which carries the genetic markers *relA1*, *mutS*, *mutT* and *mutD5*,
 - b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,
 - c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no impeding enzyme activity,
 - d) incubating this microorganism to detect the enzyme activity in at least one selection medium which comprises at least one enzyme substrate to recognize newly generated catalytic activity in the enzyme, with or without other indicator substances, and
 - e) selecting the microorganisms which show newly generated catalytic activity, said microorganisms in steps c), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts,
- wherein the enzyme is selected from the group consisting of lipases, amidases, nitrilases, ether hydrolases, peroxidases, glycosidases and phytases.

13.(previously added) The method of claim 12, wherein the enzyme is a lipase.

BORNSCHEUER et al., Serial No. 09/161,680

14.(previously added) The method of claim 12, wherein the enzyme is an amidase.

15.(previously added) The method of claim 12, wherein the enzyme is a nitrilase.

16.(previously added) The method of claim 12, wherein the enzyme is an ether
hydrolase.

17.(previously added) The method of claim 12, wherein the enzyme is a peroxidase.

18.(previously added) The method of claim 12, wherein the enzyme is a glycosidase.

19.(previously added) The method of claim 12, wherein the enzyme is a phytase.

20.(previously added) The method of claim 13, wherein the lipase is selected from the
group of lipases consisting of *Pseudomonas cepacia* lipase PS, *Pseudomonas*
cepacia lipase AH, acylase, *Rhizopus delamar* lipase, *Rhizopus javanicus* lipase,
Candida rugosa lipase, *Mucor javanicus* lipase, *Penicillium roquefortii* lipase,
Penicillium cyclopium lipase, *Chromobacterium viscosum* lipase, *Rhizomucor*
miehei lipase, *Humicola lanuginosa* lipase, *Candida antarctica* lipase B and
Candida antarctica lipase A.

21.(previously added) The method of claim 12, wherein steps (a) to (e) are performed several times in sequence by reisolating and retransforming the DNA sequence from the microorganisms selected in step (e) to the strain *Escherichia coli* XL-1 Red or its functional derivative.

22. (currently amended) A method for generating new catalytic activity in an enzyme, comprising the steps of:

- a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red or into a functional derivative thereof which carries the genetic markers *relA1*, *mutS*, *mutT* and *mutD5*,
- b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,
- c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no impeding enzyme activity,
- d) incubating this microorganism to detect the enzyme activity in at least one selection medium which comprises at least one enzyme substrate to recognize newly generated catalytic activity in the enzyme, with or without other indicator substances, and
- e) selecting the microorganisms which show newly generated catalytic activity, said microorganisms in steps c), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts,

BORNSCHEUER et al., Serial No. 09/161,680

wherein the enzyme is an esterase selected from the group consisting of *Pseudomonas fluorescens* esterase, pig liver esterase and *Thermoanaerobium brockii* esterase.

23.(previously added) The method of claim 22, wherein steps (a) to (e) are performed several times in sequence by reisolating and retransforming the DNA sequence from the microorganisms selected in step (e) to the strain *Escherichia coli* XL-1 Red or its functional derivative.